

WHAT IS CLAIMED IS:

1           1.     A method for mapping a site of post-translational modification on a  
2 post-translationally modified polypeptide, said method comprising:

3                 (a) site-specifically cleaving a peptide bond of the post-translationally  
4 modified polypeptide with an endopeptidase at said site of post-translational modification to  
5 produce a degraded post-translationally modified polypeptide; and

6                 (b) after step (a), determining said site of post-translational modification.

1           2.     The method of claim 1, wherein said post-translational modification is  
2 selected from phosphorylation, sulfonation, glycosylation, acetylation, methylations, ADP-  
3 ribosylation, methionine oxidation, cysteine oxidation, and cysteine lipidation.

1           3.     The method of claim 1, wherein said post-translational modification is  
2 phosphorylation of an amino acid selected from tyrosine, serine, and threonine.

1           4.     The method of claim 1, wherein said post-translational modification is  
2 sulfonation of a tyrosine.

1           5.     The method of claim 1, wherein said site of post-translational  
2 modification is determined by a method comprising determining the mass spectrometry  
3 fragmentation pattern of the degraded post-translationally modified polypeptide.

1           6.     The method of claim 1, wherein said endopeptidase is a serine protease  
2 comprising an active site that specifically binds to said post-translational modification.

1           7.     The method of claim 6, wherein said serine protease is subtilisin.

1           8.     A serine protease which site-specifically cleaves a peptide bond of a  
2 post-translationally modified polypeptide at a site of post-translational modification, wherein  
3 said serine protease comprises an active site that binds to said site of post-translational  
4 modification.

1           9.     The serine protease of claim 8, wherein said post-translational  
2 modification is selected from phosphorylation, sulfonation, glycosylation, and acetylation.

1                   10.    The serine protease of claim 8, wherein said post-translational  
2 modification is phosphorylation of an amino acid selected from tyrosine, serine, and  
3 threonine.

1                   11.    The serine protease of claim 8, wherein said post-translational  
2 modification is sulfonation of a tyrosine.

1                   12.    The serine protease of claim 8, wherein said serine protease is  
2 subtilisin.

1                   13.    The serine protease of claim 8, wherein said serine protease is encoded  
2 by a nucleic acid sequence that hybridizes under highly stringent hybridization conditions to  
3 a nucleic acid encoding a polypeptide comprising an amino acid sequence of Figure 1,  
4 wherein the hybridization reaction is incubated at 42°C in a solution comprising 50%  
5 formamide, 5x SSC and 1% SDS, and washed at 65°C in a solution comprising 0.2x SSC and  
6 0.1% SDS.

1                   14.    The serine protease of claim 8, wherein said serine protease comprises  
2 a subsequence having at least 70% amino acid sequence identity to an amino acid sequence  
3 of Figure 1.

1                   15.    The serine protease of claim 8, wherein said serine protease comprises  
2 a subsequence having at least 70% amino acid sequence identity to an amino acid sequence  
3 of Figure 1 and contains at least one amino acid substitution selected from P129G, E156R,  
4 S191K, G166K, and G127S.

1                   16.    The serine protease of claim 8, wherein said serine protease is encoded  
2 by an expression vector.

1                   17.    A host cell comprising the expression vector of claim 16.

1                   18.    An endopeptidase that site-specifically cleaves a peptide bond of a  
2 post-translationally modified polypeptide at a site of post-translational modification, said  
3 endopeptidase produced by a method comprising:  
4                   (a) introducing one or more point mutations to a model endopeptidase at one  
5 or more candidate amino acid positions in an active site of said model endopeptidase to

6 produce a plurality of candidate endopeptidases, wherein at least one of said plurality of  
7 candidate endopeptidases is an endopeptidase that site-specifically cleaves a peptide bond of  
8 a post-translationally modified polypeptide at a site of post-translational modification; and  
9 (b) identifying said endopeptidase that site-specifically cleaves at said site of post-translational  
10 modification.

1 19. The endopeptidase of claim 18, wherein said model endopeptidase  
2 comprises a subsequence having at least 70% amino acid sequence identity to an amino acid  
3 sequence of Figure 1.

1 20. The endopeptidase of claim 18, wherein said model endopeptidase is  
2 encoded by a nucleic acid sequence that hybridizes under highly stringent hybridization  
3 conditions to a nucleic acid encoding a polypeptide comprising an amino acid sequence of  
4 Figure 1, wherein the hybridization reaction is incubated at 42°C in a solution comprising  
5 50% formamide, 5x SSC and 1% SDS, and washed at 65°C in a solution comprising 0.2x  
6 SSC and 0.1% SDS.

1 21. The endopeptidase of claim 18, wherein said one or more candidate  
2 amino acid positions is selected from P129, E156, S191, G166, and G127.

1 22. The endopeptidase of claim 18, wherein said one or more candidate  
2 amino acid positions is P129 and said point mutation is a glycine or alanine substitution.

1 23. The endopeptidase of claim 18, wherein said one or more candidate  
2 amino acid positions is E156 and said point mutation is an arginine substitution.

1 24. The endopeptidase of claim 18, wherein said one or more candidate  
2 amino acid positions is E156 and said point mutation is a lysine substitution.

1 25. The endopeptidase of claim 18, wherein said one or more candidate  
2 amino acid positions is P129 and E156, wherein said point mutation is glycine at p129 and  
3 arginine at E156.

1 26. The endopeptidase of claim 18, wherein, before step (a), said one or  
2 more candidate amino acid positions are identified by a method comprising:

3 (i) generating a three-dimensional structure of said model endopeptidase  
4 active site;  
5 (ii) generating a three-dimensional structure of said post-translationally  
6 modified polypeptide;  
7 (iv) comparing the three-dimensional structure of said model endopeptidase  
8 active site with said post-translationally modified polypeptide, thereby identifying one or  
9 more candidate amino acid positions that, upon introduction of one or more point mutations  
10 at one or more of said candidate amino acid positions, produces a plurality of candidate  
11 endopeptidases, wherein at least one of said plurality of candidate endopeptidases is said  
12 endopeptidase that site-specifically said peptide bond of said post-translationally modified  
13 polypeptide cleaves at said site of post-translational modification.

1                           27. An isolated nucleic acid encoding a endopeptidase which site-  
2 specifically cleaves a peptide bond of a post-translationally modified polypeptide at a site of  
3 post-translational modification and which comprises one or more point mutations at one or  
4 more amino acid positions within the endopeptidase active site,

5 wherein said isolated nucleic acid hybridizes under highly stringent  
6 hybridization conditions to a nucleic acid sequence of Figure 2, wherein  
7 the hybridization reaction is incubated at 42°C in a solution comprising  
8 50% formamide, 5x SSC and 1% SDS, and washed at 65°C in a solution  
9 comprising 0.2x SSC and 0.1% SDS.

1 28. An expression vector comprising the nucleic acid of claim 27.

1 29. A host cell transfected with the vector of claim 28.

31. An expression vector comprising the nucleic acid of claim 30.

1 32. A host cell transfected with the vector of claim 30.